PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Joseph M. Penninger
Michael A. Crackower

Serial No.: 10/518,599

Filed: May 31, 2005

For: ACE2 ACTIVATION FOR TREATMENT OF HEART, LUNG AND KIDNEY DISEASE AND HYPERTENSION

Group Art Unit: 1632

Examiner: Anoop Kumar Singh

Atty. Dkt. No.: SONN:064US

CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

November 1, 2007
Date

Travis M. Wohlers

DECLARATION OF DR. MANFRED SCHUSTER UNDER 37 C.F.R. § 1.132

I, Manfred Schuster, hereby declare as follows:

1. I am an Austrian citizen residing in Vienna, Austria. I am the Head of Research and Development at Apeiron Biologics, which is the licensee of the above-referenced patent application. I have extensive research experience in the field of pharmacology. A copy of my *curriculum vitae* is attached as Exhibit 1.

2. I have reviewed the specification of the above-reference application, the current set of claims, and the Office Action dated June 1, 2007 ("the Action"). I understand that the Action rejected

claims 67-68, 73, 100-102, and 104-107 for lack of enablement. I am submitting this declaration to provide additional evidence that the current claims are enabled by the specification.

3. This declaration describes four studies on therapeutic uses of recombinant human soluble ACE2 (rhACE2) protein performed under my direction at Apeiron Biologics and in collaboration with researchers at University Hospital Innsbruck. These studies describe the use of rhACE2 in treating: (1) cardiovascular complications in mice; (2) pulmonary hypertension in pigs; (3) kidney disease in mice, and (4) acute respiratory distress syndrome in pigs.

4. All of the studies described in this declaration use a recombinant human ACE2 (rhACE2) protein. We pursued studies of the rhACE2 protein because of the disclosure in the present specification that ACE2 was a critical negative regulator of the renin-angiotensin system (RAS) (Specification, paragraph bridging pages 2-3) and that the activation of ACE2 could be used to treat hypertension, cardiac disease, kidney disease, and lung disease (Specification, p. 3, ln. 3-6). One manner in which the specification discloses that ACE2 may be activated is to administer an ACE2 protein to a subject (Specification, p. 9, ln. 16-25). The specification provides DNA and protein sequences for human ACE2 (SEQ ID NO: 1 and SEQ ID NO: 2, respectively). The specification also discloses that ACE2 is highly conserved among various organisms (see e.g., Specification, Figure 1A).

5. Based on these teachings in the specification, we produced a recombinant human ACE2 (rhACE2) protein using routine recombinant DNA techniques. The specification discloses the nucleic acid and amino acid sequences of human ACE2 (SEQ ID NOs: 1 and 2). In addition, the specification discloses the location of the extracellular domain, consensus zinc binding site, and transmembrane domain (FIG. 1 and corresponding text). In view of this disclosure, we expressed

recombinantly the extracellular domain, which includes the consensus zinc binding site, of human ACE2 (amino acid residues 1-740) in stably transfected cells. The expression product was purified to homogeneity (purity >98% measured by SEC-HPLC) by applying a capture step on a DEAE Sepharose® anion exchanger resin followed by polishing step on a Superdex® 200 gel filtration column. The specification also teaches that an ACE2 polypeptide may be contained in buffered solutions with a suitable pH and isoosmotic with physiologic fluids (Specification, p. 22, ln. 10-14). The protein preparation used in the present study was supplied in a concentration of 1.5 mg/ml in physiological buffer. This recombinant human ACE2 protein, which is referred to interchangeably in this declaration as rhACE2 and APN 01, was used in all of the studies described below.

6. All APN 01 batches were compared to the commercially available ACE2 933-ZN (R&D Systems) and to an in-house standard. Biochemical and immunological properties of all products were measured by conventional methods, such as those described in the specification at pages 22-25. In particular, the biochemical and immunoligcal properties measured by SEC-HPLC, SDS-PAGE, Western Blot and Sandwich-ELISA (using the polyclonal goat AF933 and the monoclonal MAB933 ACE2 specific antibodies (both R&D Systems) for capture and detection respectively) were almost identical. As taught in the specification ACE2 activity may be evaluated by measuring proteolytic cleavage of one or more of its substrates (Specification, p. 18, ln. 8-15). The specification also cites to Vickers *et al.* as an example of an *in vitro* assay for ACE2 (Specification, p. 18, ln. 16-19). In view of these teachings, we characterized specific enzymatic activities using the fluorescent labeled tri-peptide APK and Ang II as substrates and corresponded to respectively 5.2±0.1 $\mu mol.mg^{-1}.min^{-1}$ (reactivities 4.4 fold increased in

comparison to ACE2 933-ZN). Endotoxin concentration was measured by LAL Endochrome test and was below 2 EU/mg for all batches.

ACE2 Treatment of Cardiovascular Complications

7. By following the teachings in the present specification we have demonstrated that rhACE2 can treat cardiovascular complications in mice. As mentioned above, the specification discloses that ACE2 is a critical negative regulator of the renin-angiotensin system (RAS). In particular, ACE2 cleaves angiotensin I (Ang I) to generate Ang 1-9, and it cleaves angiotensin II (Ang II) to generate Ang 1-7 (Specification, p. 29, ln. 10-16) (*see also* Exhibit 2, Figure 1). The specification teaches that a loss of ACE2 resulted in an increase in Ang II and led to detrimental heart defects in the ACE2 knockout mouse (p. 36, ln. 14 – p. 38, ln. 26). The specification also teaches that an ACE2 decreased state, such as cardiac disease, may be treated by administering to an animal in need thereof an effective amount of an agent that can increase the expression of ACE2 (Specification, p. 9, ln. 10-15). This agent may be an ACE2 protein (Specification, p. 9, ln. 16-25). The specification further teaches that the agent may be administered to a subject via intravenous injection or intraperitoneal injection (Specification, p. 21, ln. 27-30). Accordingly, to study the effect of ACE2 therapy on cardiovascular complications, we administered rhACE2 either by intravenous injection or intraperitoneal injection to: (1) healthy Balb-c mice, (2) healthy Balb-c mice administered Ang II, (3) Balb-c mice and ACE2 knock-out Balb-c mice subjected to aortic banding heart failure, and (4) Balb-c mice subjected to coronary ligation ischemia.

8. Recombinant human ACE2 (APN 01) was administrated intravenously in a dosage from 0.1-6.4 mg/kg to healthy Balb-c mice. A control group received in parallel an equal volume of

the buffer solution alone. Hemodynamic effects were recorded online using a heart catheter implanted via the right aorta carotis through the aortic valve into the left ventricle. The rhACE2 solution was administrated via a second catheter placed into the left or right *vena jugularis externa*. Calibration was performed by administration of 5% NaCl. rhACE2 was administrated at a flow rate of 20 µl/min until the intended amount was reached. Hemodynamic parameters were recorded after 5 minutes when values stabilized. We demonstrated a cardio-protective positive inotropic effect of rhACE2: rhACE2 mediated a dose dependent reduced arterial elasticity, an increased cardiac output and a reduced end systolic pressure. The combination of all these parameters leads to a reduced heart beat rate and an increased heart efficiency as shown in Figure 2A of Exhibit 2 attached to this declaration. The decrease in systolic pressure following rhACE2 treatment is shown in Figure 2B of Exhibit 2.

9. To evaluate the ability of rhACE2 to neutralize Ang II related effects increasing concentrations of Ang II were administrated to Balb-c mice pre-treated with APN 01. Ang II was infused continuously because of its short half-life time of 10 seconds. The administered Ang II solution contained 50 ng/µl. Flow rates were kept constant for 3 minutes to reach a steady state and were further increased again. Several negative effects of continuously infused, and thus elevated Ang II, on cardiovascular parameters like reduced power or ejection fraction, increased systolic pressure, and increased end systolic volume were neutralized or significantly attenuated in rhACE2 treated mice (Exhibit 2, Figure 3). These results strongly indicate a beneficial effect of ACE2 treatment in pathological situations of elevated cardiac Ang II.

10. To evaluate the therapeutic benefit of ACE2 in chronic heart failure Balb-c mice as well as ACE2 knock-out Balb-c mice (the same ACE2 knock out mouse model disclosed in the

specification) were subjected to aortic banding. Aortic banding is a generally accepted approach for modeling chronic heart failure. Following aortic banding ACE2 knock-out mice showed severe pathologic cardiovascular symptoms such as increased left ventricular weight, increased left ventricular end systolic dimension, increased left ventricular end diastolic pressure and decreased fractional shortening. rhACE2 was administered to the ACE2 knock-out mice by intraperitoneal injection at a dosage of 2 mg/kg daily for three weeks when hemodynamic heart parameters were investigated. The rhACE2 treatment completely relieved the symptoms of aortic banding in ACE2 knock-out mice as shown in Figure 4 of Exhibit 2.

11. A therapeutic effect of ACE2 in myocardial infarction was investigated in a generally accepted ischemic-reperfusion model in Balb-c mice. Ischemia was induced by coronary ligation for 15 minutes followed by reperfusion, already in presence of rhACE2. We further administered rhACE2 as intravenous bolus daily for four weeks and analyzed several cardiac parameters by ultrasonic cardiac echography. As early as the second week of treatment statistically significant differences in fraction shortening (FS), which is an essential parameter for cardiac efficiency, appeared between treated and control animals. rhACE2 therapy led to an enhanced FS of 13%, while control animals showed values of 8%. A graphical representation is given in Figure 5. This observation confirms a therapeutic benefit of ACE2 in myocardial infarction.

12. In summary, this study demonstrated that ACE2 therapy reduced heart beat rate and increased heart efficiency, neutralized or significantly reduced the negative effects of elevated Ang II, relieved the symptoms of aortic banding in ACE2 knock-out mice, and provided a therapeutic benefit in myocardial infarction.

ACE2 Treatment of Pulmonary Hypertension

13. By following the teachings in the present specification we have demonstrated that rhACE2 can treat pulmonary hypertension in pigs. As discussed above, ACE2 cleaves angiotensin I (Ang I) to generate Ang 1-9, and it cleaves angiotensin II (Ang II) to generate Ang 1-7 (Specification, p. 29, ln. 10-16) (*see also* Exhibit 2, Figure 1). The specification discloses that ACE2 is expressed in the lung (Specification, p. 40, ln. 11-12). Loss of ACE2 resulted in increased sensitivity to lung injury in the ACE2 knockout mouse (Specification, p. 36, ln. 14 – p. 38, ln. 26). The specification teaches that an ACE2 decreased state, such as lung disease, may be treated by administering to an animal in need thereof an effective amount of an agent that can increase the expression of ACE2 (Specification, p. 9, ln. 10-15; p. 40, ln. 18-20). This agent may be an ACE2 protein (Specification, p. 9, ln. 16-25). The specification also teaches that the agent may be administered to a subject via intravenous injection (Specification, p. 21, ln. 27-30). Accordingly, we studied the effects of rhACE2 administered by intravenous injection to piglets ventilated using a hypoxic gas mixture.

14. In this study, two groups of piglets (six animals per group) were anesthetized and ventilated using a hypoxic gas mixture ($FiO_2 = 0.13$). Ventilation with a hypoxic gas mixture is a generally accepted method for inducing pulmonary hypertension. Before ACE2 therapy, mean pulmonary arterial pressure increased similarly in both groups. After 30 minutes in hypoxia, animals received either a single dose of 0.4 mg/kg rhACE2 intravenously or saline as control. Variables observed included hemodynamics (systemic arterial pressure, central venous pressure, pulmonary artery pressure, pulmonary arterial wedge pressure), pulmonary function variables assessed using the multiple inert gas elimination technique (MIGET) and a standard lead-II ECG.

While there were no differences in systemic arterial pressure or pulmonary function, the elevated mean pulmonary arterial pressure significantly decreased in animals treated with rhACE2 ($p<0.05$ at 90, 120, 150 and 180 minutes after administration). A graphical representation of pulmonary arterial pressure is presented in Figure 6 in Exhibit 2. Treatment was well tolerated without any signs of side effects or toxicity. These results indicate a therapeutic benefit of ACE2 in pulmonary hypertension.

ACE2 Treatment of Kidney Disease

15. By following the teachings in the present specification we have demonstrated that rhACE2 can treat diabetic nephropathy in mice. The specification discloses that ACE2 is expressed in the kidney (Specification, p. 35, ln. 9-10). Loss of ACE2 resulted in enhanced Ang II signaling which ultimately mediated detrimental effect in the kidneys of the ACE2 knockout mouse (Specification, p. 35, ln. 9 to p. 36, ln. 11). The specification teaches that an ACE2 decreased state, such as kidney disease, may be treated by administering to an animal in need thereof an effective amount of an agent that can increase the expression of ACE2 (Specification, p. 9, ln. 10-15). This agent may be an ACE2 protein (Specification, p. 9, ln. 16-25). The specification also teaches that the agent may be administered to a subject via intraperitoneal injection (Specification, p. 21, ln. 27-30). Accordingly, we studied the effects of rhACE2 administered by intraperitoneal injection to mice with diabetic nephropathy.

16. Heterozygous Akita mice (C57BL/6-Ins2), which develop after 3 months a diabetic phenotype of pathologic hyperglycemia, albuminuria and oxidative stress caused diabetic glomerulosclerosis, were used in this study. rhACE2 was administrated daily by intraperitoneal injection at a dosage of 2 mg/kg for 4 weeks. Kidney function was assessed by measuring

albumin excretion in urine. As shown in Figure 7, albumin excretion in urine due to kidney damage was reduced significantly compared to a control group following 4 weeks of treatment with rhACE2. These findings confirm a therapeutic benefit of ACE2 in diabetic nephropathy.

ACE2 Treatment of Acute Respiratory Distress Syndrome

17. It is my understanding that Dr. Nikolaus Neu previously provided a declaration describing the work of his research group at University Hospital Innsbruck on a study of rhACE2 (APN 01) in a piglet acute respiratory distress syndrome (ARDS) model. This study is in collaboration with myself and others at Apeiron Biologics. This study has since been expanded and is discussed below.

18. A physiological formulation of rhACE2 (APN 01) was administered to piglets (average weight = 22 kg) in an LPS-induced, randomized and placebo controlled ARDS model at the animal intensive care premises of the University Hospital Innsbruck. The piglet ARDS model is a generally accepted animal model for the study of acute respiratory distress syndrome. 11 animals were treated in a therapeutic setup with APN 01 at a dosage of 0.4 mg/kg and compared to a vehicle control group composed of 15 animals. 6 animals of each group were considered as evaluable based on their pre-treatment hemodynamic status. All animals had exactly the same age, similar body weight and had the same genetic antecedents.

19. Piglets were anesthetized for the whole experiment, intubated and ventilated (30% O_2) via the same instruments and machines used in human intensive care medicine. A central venous heart catheter was set at the *vena cava superior* to measure various heart parameters, which were visualized using an intensive care heart monitor. This central venous lock was also used to obtain venous blood samples and to deliver systemically lipopolysaccharide (LPS) as well as

APN 01. A further arterial lock was set in the *arteria femoralis* near the left leg of the animal to obtain arterial blood samples. Animals were laid ventral side down to keep them in the most physiological position for the time span of the experiment. Blood samples were investigated every 30 minutes for blood gas content, pH and Na^+, K^+ and Cl^- ion concentration. MIGET analysis was performed at one hour intervals.

20. ARDS was induced by continuous infusion of 8 μg/kg.h LPS using an automatic infuser pump. Additional 1 - 3 LPS bolus injections of up to 10 μg/kg were administered to each animal depending on individual hemodynamic parameters (PAP, SP, HF, pO_2). Nearly identical therapeutic start conditions were adjusted for all individuals by varying frequency and intensity of LPS bolus injections. The treated group consisting of 11 animals received 0.4 mg/kg APN 01 as bolus injection while the control group composed of 15 animals obtained a vehicle-only injection. Blood samples were taken at time points -120, 0, 30, 90 and 150 minutes to measure blood gas content as well as pharmacokinetic and pharmacodynamic parameters. Plasma and serum samples supplemented with a protease inhibitor cocktail were stored frozen at -80°C until analysis. The LPS and APN 01 dosage scheme as well as plasma and serum sampling time points are shown in Figure 8 in Exhibit 2. Animal weight and LPS dosage parameters are summarized in Figure 9 in Exhibit 2. Body weight and cumulative LPS doses were equally distributed over both groups ($p>0.7$ or >0.1 and $p>0.8$ or >0.7 by t-test for LPS and body weight in all animals or selected individuals, respectively).

21. The enzymatic activity of rhACE2 in body fluids was measured by its ability to cleave the fluorescent peptide substrate Mca-Ala-Pro-Lys(Dnp)-OH. In brief, serum samples were frozen immediately and stored at -20°C until analysis without the addition of complexing supplements

or protease inhibitors. Cleavage was measured in 1:2 and 1:10 diluted samples (final assay dilution) using excitation and emission wavelengths of 320 and 430 nm respectively in presence of 100 μM substrate in 50 mM MES, 300 mM NaCl, 10 μM $ZnCl_2$ and 0.01% Brij-30 at pH 6.5. Evaluation was performed by comparing the maximal initial slope of the fluorescence/time curve to respective maximal initial slopes of a serial rhACE2 dilution in normal piglet serum composed of 7 concentrations equally distributed between 0.05 and 1 μg/ml.

22. Ang II titers in piglet plasma samples were measured using the Ang II Enzyme Immunoassay Kit A05880 (SPI-BIO, Montigny Le Bretonneux, France) according to the manufacturers instructions. In brief, plasma samples were immediately pre-treated with a protease inhibitor cocktail composed of Phenanthroline, Pepstatin A, p-hydroxy-mercuribenzoic acid and EDTA and stored at -20°C until analysis. Ang II was captured by an anti-Ang II monoclonal antibody immobilized on a microtiter plate and covalently linked to the plate by glutar-di-aldehyde. After intense washing and denaturation Ang II reacts with an acetylcholin-esterase (AChE)-labeled monoclonal antibody as tracer. AChE reacts with Ellman's reagent to form a colored reaction product whose intensity is measured photometrically at wavelength 405 nm and used for quantification. Evaluation was performed by comparing the optical densities of the sample to a serial Ang II dilution in normal piglet plasma composed of 9 concentrations equally distributed between 0 and 250 pg Ang II /ml.

23. Kidney parameters - creatinin, urea, potassium and phosphate levels, were measured in serum samples at the Veterinary University Vienna according to their standard operating procedures.

24. The results are expressed as the mean±SEM. Statistical analysis was performed by unpaired t test. The level of significance was set at $p<0.05$.

25. The APN 01 administration was well tolerated and did not show any apparent side effects such as a dramatic blood pressure drop or heart frequency increase in any of the treated animals. Figure 10 in Exhibit 2 shows a graphical representation of mean arterial pressure (MAP) normalized on individual baselines at the start point of the therapy. Data of all animals (dashed lines) as well as results of animals that survived the whole experiment (solid lines) are displayed. No statistically significant differences to the respective control groups were observed.

26. ACE2 resorbtion and distribution were investigated in several body liquids by measuring ACE2 activity in serum, ascites, lung lavage liquid and urine. We calculated ACE2 equivalent concentrations based on a calibration curve. ARDS was induced in all animals by continuous LPS administration. Figure 11 in Exhibit 2 shows the time dependency of ACE2 titers in serum samples of animals receiving 0.4 mg/kg APN 01 in comparison to the vehicle only control group. No intrinsic ACE2 activity was found in any baseline serum samples or in any samples of the vehicle control group. Detectable ACE2 activities appeared immediately after the APN 01 bolus administration at the start point of the therapy started at 0 minutes. A maximal serum concentration of 5.0±0.8 µg/ml was attained 30 minutes after administration. This amount correlates with the expected value assuming a total serum volume of 1.0 liter for an animal of 25 kg body weight (0.08 x 25 x 0.5) which has received 10 mg rhACE2. This should lead to calculated concentrations of nearly 10 µg/ml if 100% of administrated biologic would be available in the circulation. ACE2 serum concentrations decreased steadily until the end of the experiment during the following 150 minutes to 64% of the initial values.

27. We investigated the presence of ACE2 in several body liquids and found significant ACE2 activity in ascites, lung lavage and urine in all animals having received APN 01. A graphical representation is shown in Figure 12 in Exhibit 2. Lung lavage showed an important ACE2 activity corresponding to 2 μg/ml. We did not detect ACE2 activity in baseline urine while urine at the end of the experiment also showed measurable ACE2 titers of 1 μg/ml. ACE2 activity in ascites attained similar levels.

28. We further investigated APN 01 pharmacokinetics and pharmacodynamics at the identical dosage in 3 healthy animals. This study was run under exactly the same conditions: piglets were also anesthetized and intubated to serve as identically treated healthy controls. Manipulation and observation time had to be kept below 6 hours due to practical and ethical reasons. APN 01 was administrated at the beginning of the experiment at 0.4 mg/kg and its activity was measured in harvested serum samples. Figure 13 in Exhibit 2 displays the time dependency of rhACE2 activity in a logarithmic (base 2) representation. ACE2 activity increased from no activity prior to administration to a maximum value of an ACE2 equivalent concentration of 5.1±0.2 μg/ml 30 minutes after administration. Activity decreased to 42% in 300 minutes. The observation time of 6 hours still is very short to exactly determine ACE2 serum half-life time. The initial alpha distribution still remains predominant. An approximated half life time of at least 5.1 hours nevertheless can be estimated for the beta-distribution.

29. Ang II is one of the predominant biologically active members of the RAS and acts as a potent vasoconstrictor. It is also an important substrate for ACE2. We, therefore, expected that the administration of enzymatically active ACE2 would directly affect Ang II levels and activate counter-regulation mechanisms of RAS. We also expected effects of LPS administration on Ang

II titers. We measured Ang II titers to verify these expectations. Ang II titers were measured in plasma samples collected in parallel to serum samples for ACE2 activity quantification (Figure 14). First, Ang II levels were investigated in plasma samples of one healthy piglet receiving one bolus injection of 0.4 mg/kg APN 01 and subsequently in piglets included in the ARDS model. Basal Ang II titers were very similar in all animals and groups and corresponded to 20±6 pg/ml. Ang II titers decreased immediately after APN 01 administration in samples of three healthy animals and attained a minimal value of 3 pg/ml after one hour. Concentration stabilized on this level for the following two hours. It seems that counter-regulatory mechanisms took place to restore Ang II titers in presence of functional rhACE2. Titers increased from then on to a value of 10 pg/ml 2.5 hours after APN 01 delivery and to a concentration of 14 pg/ml 3.5 hours after the administration. In animals of the ARDS model, LPS administration strongly influenced Ang II titers. After a "lag phase" of one hour after the first LPS administration titers increased rapidly and attained a plateau value of 150±79 pg/ml two hours after beginning LPS delivery. Ang II concentration increased further to 327±107 pg/ml until the end of the experiment for all animals receiving the vehicle formulation. In contrast, Ang II titers decreased rapidly for animals treated with rhACE2 to 50 pg/ml 30 minutes after ACE2 bolus administration. The basal Ang II level of 20 pg/ml was attained in this group 2.5 hours later after ACE2 injection. Titers interestingly did not fall below this value although ACE2 activity was measured. These results are shown in Figure 14 in Exhibit 2.

30. Pulmonary arterial pressure (PAP) was measured online during the whole study and is summarized in Figure 15 in Exhibit 2. Both groups started at values of 25±1 mmHg, which corresponds to the pressure measured also in healthy individuals. PAP increased during the

experiment in both groups from the start of continuous administration of LPS. Values increased steadily and reached values around 40 mmHg when ARDS was diagnosed (0 minutes) and the therapy was initiated. PAP slightly increased further in both groups for 30 minutes to 45 mmHg. Values from control and treated animals were very similar until this point (t-test: $p>0.65$). Curves began to separate 30 minutes after initiation of the therapy: PAP of animals in the control group further increased while values of APN 01 treated animals continuously decreased to values below 40 mmHg. The difference between both groups was statistically significant at $p<0.05$ for all time points 30 minutes post therapy and significant at $p<0.01$ 150 minutes post therapy.

31. Arterial oxygen concentration directly correlates with lung function and efficiency. Oxygen concentration was measured in arterial blood samples taken every 30 minutes from APN 01 treated LPS-induced ARDS pigs and control pigs. Values of all evaluable animals are displayed in Figure 16A in Exhibit 2. Baseline blood oxygenation was in the expected range, >90 mmHg, for all animals displayed in Figure 16A. Animals that did not survive the study or whose arterial oxygen tension started below 90 mmHg or dropped to rapidly during the preparation of the study were excluded from this evaluation. A representation of all values without exclusion of any individual is given later. Oxygen concentration decreased dramatically from 92 to 60 mmHg in arterial blood in both groups from the time point of LPS infusion. Lung functions at this time point were already severely affected by ARDS and animals would not survive without intubation and mechanical ventilation. Oxygen concentration decreased further in animals of the control group down to 50 mmHg while, in contrast, a stabilization and even an increase of oxygen levels was seen in the APN 01 treated group immediately after administration. The difference between both groups was statistically significant at 120 ($p<0.04$), 150 ($p<0.01$)

and at 180 minutes ($p<0.01$) after APN 01 infusion. APN 01 or placebo also were administered in a therapeutic setup to piglets in a mechanical lung damage (meconium aspiration) ARDS model. Following treatment with APN 01, arterial partial oxygen tension stabilized and statistically significant increased in the treated group ($p<0.01$, Figure 16B).

32. We further evaluated the individual effect of APN 01 administration by normalizing oxygen tensions on individual baselines (100%) at the time point 0 minutes when therapy began. A graphical representation of evaluable animals as well as all individuals is given in Figure 17 in Exhibit 2. Oxygen tension decreased for placebo while values nearly stabilized on baseline levels or increased for included animals receiving APN 01. The difference between both groups becomes statistically significant for all time points 120 minutes post therapy (as well as for the cohort including all animals at 60 and 120 minutes post therapy).

33. Logarithmic SD of perfusion distribution is a calculated parameter that inversely correlates with lung efficiency. Log SDQ was calculated for both animal groups and is represented graphically in Figure 18 of Exhibit 2. Baseline levels are similar for both animal cohorts. The values also slightly increased equally from the start. The value stabilized at 0.47 for the APN 01 treated group 30 minutes after ACE2 administration and further decreased until the end of the study. The control group in contrast showed a steep increase to values of 0.62 after ARDS diagnosis and administration of saline, stabilized on this level for the next 30 minutes and than slightly decreased. The difference between both groups was statistically significant at 90 ($p<0.05$) and at 180 minutes ($p<0.05$) after APN 01 infusion.

34. Kidney function was evaluated by measuring protein content in urine samples taken at the beginning and at the end of the study. Low protein concentrations, which would not be

detectable using standard urine test, were measured using the Bradford method. Protein levels increased from 25±2 to 39±7 μg/ml in the control group and decreased from 39±5 to 33±6 μg/ml in the APN 01 treated cohort. The relative change in relation to baseline levels was calculated individually and compared between both groups and represented in Figure 19 in Exhibit 2. APN 01 treated animals showed a statistically significant reduction ($p<0.02$) of protein concentrations (85%) at the end of the experiment in comparison to the control group (153%).

35. To further evaluate kidney function, potassium, phosphate, urea and creatinin titers were measured in all serum samples. A graphical representation of these parameters is shown in Figure 20 in Exhibit 2. The urea concentration nearly remained constant for all animals during the whole experiment and ranged between 16 and 20 mg/dl. No difference between treated and control animals could be seen. Creatinin levels slightly increased in both groups from an initial value of 0.75 mg/dl in the first phase of the study from -120 to 0 minutes. Titers of treated animals stabilized on a value of 0.8 mg/ml and then slightly decreased after APN 01 administration while the creatinin levels increased in the control group. This trend was statistically not significant. Potassium titers increased in both groups from the start of the experiment from 3.8 to 4.8 mmol/l. Both animal cohorts behaved identically from -120 to 90 minutes. Only the last sample taken at 150 minutes showed lower potassium titers in the APN 01 treated group (4.6 mmol/l) in comparison to control animals (5.2 mmol/l). This difference was statistically not significant. Phosphate titers were nearly stable at 2.60 mmol/l in both groups from the start of the study until the start of therapy. ACE2 treated animals showed a statistically significant ($p<0.05$) lower phosphate concentration 30 minutes after APN 01 delivery in comparison to the control group. These treated animals stabilized on a value of 2.55 mmol/l

while titers of the control group decreased slowly to finally end up at similar levels at the end of the experiment.

36. The ARDS animal model described here provides reproducible conditions in which to evaluate the effects of administrated drugs. Animal weight, hemodynamic parameters after ARDS induction and administered LPS quantities were similar or equally distributed in both animal groups meaning that the study design enables a valid interpretation of the observations and a statistical evaluation in comparison to a control group. It must be noted nevertheless that some animals died during the ARDS induction period, others immediately after the therapy started regardless if APN 01 or saline were administrated. Some other animals showed immediately after induction of ARDS inferior or even "free falling" hemodynamic parameters. We therefore evaluated the complete data set including all animals as well as a subset of animals that fulfilled the following inclusion parameters: survival for at least 90 minutes after induction of ARDS as well as a paO_2 >90 mmHg when therapy was started. Both evaluations are presented and discussed here.

37. In summary, the results of the study in the ARDS piglet model demonstrate the therapeutic benefit of ACE2 therapy. In particular, this study demonstrated that ACE2 therapy increased lung function and improved kidney function in the ARDS piglet model.

Administration of Telmisartan in the Piglet Acute Respiratory Distress Syndrome Model

38. As discussed above, systemic administration of APN 01 demonstrated a therapeutic benefit to treat ARDS in an LPS-induced sepsis model in piglets. To investigate whether the observed therapeutic benefit of an ACE2 therapy in ARDS is mainly mediated by the reduction of Ang II titers, the increase of Ang 1-7 titers, or both. We studied whether the blockade of the

AT1 receptor by Telmisartan is sufficient to reproduce the therapeutic effects we observed with APN 01 and if a combination of the blockade of AT1 together with the administration of APN 01 would even increase these therapeutic effects. As illustrated in Figure 1 in Exhibit 2, AT1 is a receptor for AngII whereas Mas is a receptor for Ang 1-7.

39. Telmisartan (Figure 21a in Exhibit 2) (2-[4-[[4-methyl-6-(1-methylbenzoimidazol-2-yl)-2-propyl-benzoimidazol-1-yl]methyl]phenyl]benzoic acid (IUPAC)) is an AT1 receptor blocker. The active compound was extracted and purified from the drug formulation Micardis® (Boehringer Ingelheim, lot number 604260). Tablets were ground mechanically and solubilized into 100 mM NaOH to extract the active drug component. Insoluble components of the drug formulation were separated by centrifugation at 6000 x g, 4°C for 20 minutes. The clear solution containing Telmisartan was neutralized by drop wise addition of 3.6M HCl and Telmisartan was precipitated while keeping the solution under agitation. The precipitated product was separated by centrifugation. The product was dissolved again in 100 mM NaOH and 15% Mannitol (w/v) was added. The clear solution was adjusted to pH 8.2 by drop wise addition of 3.6M HCl. RP-HPLC analysis of the product is shown in Figure 21b. Telmisartan had a concentration of 12.8 mg/ml and a purity of > 93%.

40. Piglets were anesthetized for the whole experiment, intubated and ventilated at 30% O_2 via the same instruments and machines used in human intensive care medicine. A central venous heart catheter was set at the *vena cava superior* to measure various heart parameters, which were visualized using an intensive care heart monitor. This central venous lock was also used to obtain venous blood samples and to deliver systemically LPS as well as Telmisartan or APN 01. A further arterial lock was set in the *arteria femoralis* near the left leg of the animal to obtain

arterial blood samples. Animals were laid the ventral side down to keep them in the most physiological position for the time span of the experiment. Blood serum and plasma samples were taken every 30 minutes for further analysis and stored at -20°C.

41. All animals (twins) had exactly the same age, similar body weights and had the same genetic antecedents. Body weight and cumulative LPS doses were equally distributed over both groups (t test). Animal numbers, group assignment, body weights as well as administrated cumulative LPS quantities are summarized in Figure 23 in Exhibit 2.

42. Telmisartan was administrated to the piglets in this study at the usual human dosage of 1 mg/kg. This dosage has been investigated in humans following oral as well as intravenous delivery, without severe side effects. Animals in the present study, however, already suffered from ARDS and their hemodynamic status was unstable when therapy begun. Bolus intravenous administration of Telmisartan led to an abrupt blood pressure drop. This destabilized hemodynamic parameters and led to immediate animal death. We, therefore, administered Telmisartan intravenously at a dosage of 1 mg/kg dissolved in 100-200 ml autologous blood at 10-15 ml/min. Blood pressure dropped also and had to be maintained by administration of phenylephrine. Phenylephrine was injected when MAP dropped below 50 or SAP below 40 mmHg.

43. ARDS was induced in piglets using the dosage scheme depicted in Figure 22 in Exhibit 2. LPS was continuously administrated systemically to all animals at an infusion rate of up to 8 μg/kg.h using an automatic infuser pump. Additional 1-3 bolus LPS injections of up to 10 μg/kg were administrated to each animal depending on individual hemodynamic parameters (PAP, SP, HF, pO_2). Nearly identical therapeutic start conditions were adjusted for all individuals by

varying frequency and intensity of LPS bolus injections. The Telmisartan treated group (15 animals) received 1 mg/kg Telmisartan as intravenous injection. Telmisartan was dissolved in 100-200 ml autologous blood, which was collected immediately before the experiment and anti-coagulated with Heparin. The solution was infused at a flow rate of 10-15 ml/min. Blood samples were taken at time points -120, 0, 30, 90 and 150 minutes (indicated in Figure 22) to measure blood gas content as well as pharmacokinetic and pharmacodynamic parameters. Plasma samples supplemented with a protease inhibitor cocktail were stored frozen at -80°C until analysis.

44. ACE2 resorbtion and distribution were investigated in several body liquids by measuring the time dependent ACE2 activity in serum, ascites, lung lavage liquid and urine. Results are displayed in Figure 24 in Exhibit 2. ACE2 activity was detected only in animals that received APN 01 or the APN 01/Telmisartan combination. No baseline ACE2 activity was found in pre-treatment samples or in samples of control animals. In APN 01 treated animals, a considerable ACE2 activity was measurable in serum samples for the whole duration of the therapy. The maximal serum activity was attained 30 minutes after intravenous administration of APN 01 at 0.4 mg/kg in combination with Telmisartan and was 4.8±0.4 µg/ml. This value fits with a rough estimation that nearly 10 mg ACE2 were delivered to an animal of 25 kg and would have been resorbed into a total of 1.2 l of serum per animal leading to a serum level of 8 µg/ml. Nearly the whole amount tends to be resorbed into the circulation in the first phase of the therapy. The activity further decreased during the following 2.5 hours to 48% of the maximum value. The initial ACE2 activity correlated perfectly with levels measured in samples of animals which received APN 01 alone (5.0±0.8µg/ml). Clearance of ACE2 apparently was accelerated in

animals that received a co-administration of Telmisartan. Tissue permeability appeared superior in this cohort leading to an important ACE2 activity in ascite samples but not in urine and lung lavage liquid.

45. Ang II titers were measured in plasma samples during all phases of the study. Results were compared to data obtained for animals treated exclusively with APN 01 and to control animals. A graphical representation is shown in Figure 25 in Exhibit 2. Baseline Ang II titers of 18.1±2.0 pg/ml were very similar for all individuals. Levels increased considerably following LPS administration to an average value of 64.3±21.9 pg/ml. ARDS was diagnosed at this time-point and the therapy was initiated. Previous data have shown a decrease of Ang II titers to baseline levels when animals were treated with 0.4 mg/kg APN 01. In contrast Ang II levels remained stable at elevated levels in the control group. Ang II titers in the cohort treated with Telmisartan showed the same trend observed for the previous control group. Values increased to a plateau value of 225 pg/ml and stagnated at this elevated level until the end of the experiment. Animals receiving the APN 01/Telmisartan combination showed a rapid decrease of Ang II levels down to baseline titers 30 minutes after APN 01 bolus delivery, while Telmisartan administration was still in progress or just completed. Ang II concentration did not stabilize at baseline as observed for the APN 01 group. Titers increased again for the last phase of the experiment to high levels, similar to the ones measured in control animals or in the Telmisartan cohort. Interestingly Ang II concentration of a single healthy animal that received Telmisartan at the same dosage without the induction of ARDS decreased below baseline after Telmisartan administration and remained at this low level for the following 2 hours.

46. ARDS is diagnosed as soon as paO_2/FiO_2 drops below 200 mmHg. Arterial oxygen tension is therefore the central hemodynamic parameter that directly correlates with lung efficiency and inversely with disease progression. We normalized paO_2 values, which were recorded in 30 minute intervals, on individual baseline values at the start point of the therapy and compared them to animals previously treated with APN 01 or saline. Results are shown in Figure 26. Oxygen tension only stabilized for APN 01 treated animals and decreased dramatically in all other groups. Oxygen tension of Telmisartan and saline (control group) treated animals behaved very similar and dropped to less than 85% during 180 minutes. Values of animals receiving the APN 01/Telmisartan combination surprisingly decreased even faster and reached values below 70% at the end of the experiment.

47. Pulmonar arterial pressure (PAP) was also measured in 30 minute intervals and is displayed, also normalized on individual baselines, in Figure 27. PAP increased during ARDS and only APN 01 treated animals showed a statistically significant stabilization and even a decrease of pulmonary arterial pressure in comparison to control animals. PAP increased first in the Telmisartan treated cohort, then dropped below baseline and re-increased until the end of the experiment. In the combination therapy group, PAP also decreased first to baseline, did not stabilize and increased further to values higher than 125%. It must be noted again that animals who received Telmisartan also received multiple doses of phenylephrine to stabilize MAP and to keep them alive until the end of the study. The observed effects might correlate with phenylephrine administration.

48. Mean arterial pressure (MAP) was also monitored online. Values normalized on baseline levels are displayed in Figure 28 in Exhibit 2. MAP was markedly influenced by Telmisartan

administration. Curves of Telmisartan treated animals dropped several times below values recorded for control animals. Animals were kept alive by subsequent phenylephrine administration, which biased measured MAP values. APN 01 administration, in contrast, did not appear to influence blood pressure.

49. This study was designed to elucidate if the observed therapeutic effects of ACE2 treatment were related to reduced Ang II signaling via AT1 receptor, which was blocked in this study by Telmisartan, or if other, AT1-independent effects were responsible for the therapeutic benefit. Such AT1-independent effects might be triggered by Ang 1-7, by up-regulation of ACE2 activity, by down-regulation of ACE activity or others. Ang II titers increased in our ARDS model, as expected from the inflammatory cytokine related down-regulation of endogenous ACE2 and linked accumulation of its substrate Ang II. The administration of Telmisartan did not decrease Ang II titers, which remained elevated until the end of the experiment. This leads to even further accumulation of liquid in the lung, reduces lung efficiency and worsens lung function. The exclusive administration of Telmisartan did not exert an amelioration of lung parameters and did not show any therapeutic potential to treat ARDS. It rather destabilized hemodynamics in treated animals. Thus, the therapeutic benefit of an ACE2 therapy was apparently not mediated only by the reduction of AT1 related signaling caused by lowered Ang II titers.

50. The combined administration of APN 01 and Telmisartan also did not improve ARDS symptoms. The results showed that APN 01 in combination with Telmisartan was not able to decrease the elevated Ang II titers during the whole experiment. It is possible that Telmisartan inhibits ACE2 activity in serum or that another effect led to an up-regulation of ACE activity.

The administration of phenylephrine might also influence ACE2 activity or ACE expression. ACE2 nevertheless was still in the circulation and showed full enzymatic activity, but the concentration might not be able to overcome increasing amounts of Ang II. Systemic tissue permeability, which correlated directly with Ang II titers, was higher in animals that received the combination therapy compared to those treated with APN 01 alone. This could explain the enhanced tissue permeability we observed in the combination cohort, which led to increased ACE2 serum clearance and to the elevated ACE2 concentration in ascites. Another explanation for the elevated Ang II titers in Telmisartan treated animals is a higher affinity of the drug for AT1 receptor in comparison to Ang II. Telmisartan, therefore, competes with Ang II for AT1 and displaces bound Ang II, which is delivered to the blood circulation and leads to increasing Ang II titers. Finally, it should be noted that we only investigated a single Telmisartan dose. Lower drug concentration or other AT1 receptor blockers may lead to different results. The administration of Telmisartan nevertheless appeared contra productive in our ARDS model in piglets.

Summary

51. The studies described above demonstrate that a therapeutically effective amount an ACE2 polypeptide has a beneficial effect in treating cardiovascular complications, pulmonary hypertension, kidney disease, and acute respiratory distress syndrome. These beneficial effects were demonstrated in two mammalian species, mice and pigs. Moreover, the beneficial effects achieved in mice and pigs resulted from administering a human ACE2 polypeptide. Thus, in view of these results from two different mammalian species administered an ACE2 polypeptide from a third mammalian species, one would expect that ACE2 decreased states could similarly be treated in any mammal.

52.I declare that all statements made of my knowledge are true and all statements made on the information are believed to be true; and, further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereupon.

Date: 20.10.2007

Manfred Schuster

EXHIBIT 1

CURRICULUM VITAE

Manfred Schuster

Personal data

Title	VP Research and Development CSO
Address	Josef -Weilandstrasse 84, A-2191 Schrick manfred.schuster@apeiron-biologics.com
Nationality	Austria

Education

1978-1990	Lycée Français de Vienne, Baccalauréat 1990, scientific section
1990-1997	University education: Chemistry, Biochemistry, Technical chemistry and Biotechnology, University and Technical University of Vienna, Austria

Degrees

1996	Chemistry and Biochemistry, First Degree, University of Vienna
1997	Biochemistry, Biotechnology Dipl.-Ing. (Master of science engineer) Technical University of Vienna Masters Thesis: **Optimization of an ABE-Fermentation**
2000	Biochemistry, Biotechnology Dr. nat. techn. University of Natural Resources and Applied Life Sciences, Vienna Thesis: **Establishment of a high throughput protein expression system in Yeast**

Qualifications and experience

- CSO and Head of Research and Development at Apeiron Biologics
- Expert in Molecular Biology, focused on protein, enzyme and especially antibody expression in pro- and eukaryotic expression systems, 11 years in practice
- Trained biochemist and biotechnologist with strong expertise in protein chemistry, enzymology and downstream processing
- Trained Project Manager

- Experienced working group leader (up to 6 FTE)
- Industry and drug development experience: 3 years Novartis (Genetics unit), 6 years igeneon (Immunotherapy of Cancer), 2 year CSO at Apeiron Biologics
- "Start up", laboratory and company establishment experience
- Fluent in English, French (Baccalauréat Francais), German

Professional achievements and tasks

09/2005 – actually Apeiron Biologics, Vienna, Austria

- **CSO and Head of Research and Development:**

Responsible for the research and development program of three programs
  - Enzyme substitution / enhancement therapy for lung, cardio-vascular and kidney diseases
  - Endogenous pain therapy
  - Immuno-modulation

Head of laboratory and of the scientific staff, company and laboratory establishment

- **Bio-safety Officer** and head of the bio-safety committee

03/2003 – 08/2005 igeneon Immunotherapy of Cancer, Vienna, Austria

- **Program Manager:** Project-champion and responsible for the preclinical development of a Lewis Y-specific therapeutic monoclonal antibody with enhanced effector functions
- **Head of Molecular Biology**
- **Head of a laboratory working group**
- **Bio-safety officer, member of the Key-personnel and Management**

01/2001 – 03/2003 igeneon Immunotherapy of Cancer, Vienna, Austria

- **Project Leader:** Responsible for the development of the cancer vaccine IGN101: Preclinical research, clinical (GLP) analytics until Phase II, establishment of a GMP production process, GMP production, product analytics, product stability, method development, method validation, clinical analytics, bio-assay development
- **Head of a laboratory working group** comprising five employees responsible for Molecular Biology, Protein Expression, Protein Chemistry, Protein Analytics, Cell Line Development, Cell Culture, Chromatography, Up-scaling and Bio-assay Development (ELISA, BIAcore, FACS, ADCC, CDC, PCR)
- **Member of the company Management**
- **Laboratory-Planning,** arrangement and coordination of the construction project for the actual facilities
- **Bio-safety Officer** and head of the bio-safety committee

03/2000 - 01/2001 igeneon Immunotherapy of Cancer, Vienna, Austria

- **Postdoctoral fellow:** Responsible for recombinant protein expression in pro- and eukaryotic expression systems, development of patent free expression constructs, responsible for assay development and preclinical studies
- **Head of a laboratory working group** comprising two employees

04/1997 - 03/2000 Novartis Research Center, Vienna

- **PhD student:** Genetics Unit, **Establishment of a high throughput protein expression system in Yeast**

04/1996 - 03/1997 Technical University Vienna

- **Diploma student**

Annex 1: Competences

Organization and Management

- Project Management course (Primas, 2004)
- Employee Leadership course (2004)
- cGMP course for biotechnological products (2002)
- Bio-safety Officer and head of the bio-safety committee
- Responsible for the establishment and for the operation of a laboratory and facility monitoring- and alarm-system, technical troubleshooting
- Working Group Leader: scientific administration, supervising, assay -planning, -realization, -interpretation and -documentation
- Establishment and maintenance of laboratory and facility infrastructure
- Contacts to authorities
- Health, safety and environmental protection, establishment of a safety concept

Technical competence

- Qualification for operation, training, development, validation and assessment of listed techniques
- *Molecular Biology:* DNA/RNA techniques (siRNA, Sequencing, PCR, RT-PCR, cloning, expression, ligation, enzymatical digestion, transformation, cDNA production, RNA preparation,...), protein expression in multiple pro- and eukaryotic expression systems, generation of single chain Fv by subtractive panning of a phage library
- *Chromatography and Downstream processing:* HPLC / FPLC, IEX, Affinity chromatography, SEC, RPC, HIC, Generation of chromatography matrices
- *Protein chemistry / Protein analytics / Immunology:* Western Blot, SDS-PAGE, IEF, sequencing, Dot blot, protein characterization, determination of affinity, immuno-precipitation, ELISA, enzymology, labelling, coupling, endotoxin determination (LAL), Luminex, FACS, SPR (BIAcore)
- *Cell culture / Fermentation:* Cultivation of pro- and eukaryotic cell lines, prokaryotic fermentations, eukaryotic fermentations until 10 L
- *Cellular assays:* ADCC, CDC, ELISPOT, cell proliferation

- IT competence: Software package MS-Office (Word, Excel, Powerpoint, Outlook, Internet Explorer), MS Project, Delta-Graph, Corel Draw, GraphPad, Sigma Plot, Sigma Stat, MedCalc, Auto Assembler, Chromas, Gene Runner, SlideWrite, EndNote, Unicorn, Chromeleon,...

Other qualifications

Skilled first aid man (AUVA, 2004), driving license since 1990, lifeguard training, fulfilled military service

Annex 2 – invited speaker at international congresses and symposia

2008, Protein Therapeutics, CHI Conference, San Diego, California, USA
Development of an ACE2 Enzyme Substitution Therapy

2005, Biochromatographietag, Vienna, Austria
Isolierung und Charakterisierung von Isoformen rekombinanter Antikörper

2005, Protein Therapeutics, CHI Conference, San Diego, California, USA
Development in Cancer Immunotherapy: From a murine to a humanized and finally to a glyco-engineered monoclonal antibody with enhanced effector functions

2004, Cancer Immunotherapeutics, CHI Conference, Boston, Massachusetts, USA
Increased effector functions of a monoclonal antibody by glycoform engineering, recent results.

2004, 5th European Symposium on Biochemical Engineering Science, Stuttgart, Germany
Increased effector functions of a monoclonal antibody by glycoform engineering

2003, Antibody Production and Downstream Processing, IBC conference, Basel, Switzerland
Increased effector functions of a monoclonal antibody by glycoform engineering

2002, Äkta User Seminar, Emmendingen, Germany
Automated sequential affinity chromatography

2000, 5th Interlaken Conference on Advances in Production of Recombinant Proteins, Interlaken, Switzerland
Comparison of two high throughput expression strategies in S.cerevisiae

1999, 19th International Symposium on the Separation of Proteins, Peptides and Polynucleotides, Delray Beach, Florida, USA
High throughput protein expression in Yeast; comparison of two expression strategies

1998, 2nd European Symposium on Biochemical Engineering Science, Porto, Portugal
Expression Strategies for Functional Genomics

Annex 3 – Poster Presentations

ASCO 2002
Murine monoclonal antibody 17-1A used as vaccine antigen (IGN101): Direct induction of anti-EpCAM antibodies by vaccination
Manfred Schuster, Hans Loibner, Evelyne Janzek, Gottfried Himmler, Jungbauer Alois, Rainer Hahn, Astrid Dürauer, Hellmut Samonigg

AACR 2002
Qualitative and Quantitative Dissection of the Immune Response to the Cancer Vaccine Candidates IGN101 and IGN301
Guenter Waxenecker, Gottfried Himmler, Manfred Schuster, Thomas Putz, Erich Wasserbauer, Evelyne Janzek, Renate Ohler, Stefan Stranner, Hans Loibner, Hellmut Samonigg

Treatment of Breast Cancer Patients with the Cancer Vaccine IGN101 that Induces an Immune Response against the Pan-Carcinoma Glycoprotein EpCAM
Hellmut Samonigg, Hans Loibner, Manfred Schuster and Gottfried Himmler

ASCO 2003
Phase II trial to explore the influence of concomitant chemotherapy on the immunogenicity of the cancer vaccine IGN101 in patients with epithelial cancers
H. Samonigg, G. Hofmann, T. Bauernhofer, M. Balic, H. Stoeger1 , G. Himmler, M. Schuster, F. Rosenkaimer, F. Groiss, H. Loibner

Murine monoclonal antibody 17-1A used as vaccine antigen (IGN101): Direct induction of anti-EpCAM antibodies by vaccination of cancer patients
Manfred Schuster, Stefan Stranner, Evelyne Janzek, Hans Loibner, Gottfried Himmler, Hellmut Samonigg

Vaccination with alum-adsorbed antibodies against EpCAM directly induces anti-EpCAM antibodies
Manfred Schuster, Hans Loibner, Evelyne Janzek, Gottfried Himmler, Marija Balic, Guenter Hofmann, Hellmut Samonigg

AACR 2003
Lewis Y / EpCAM co-expression in breast cancer is correlated with poor prognosis
Guido Sauter, Manfred Schuster, Gottfried Himmler, Hans Loibner

Eurocancer 2003
Expression d'EpCAM dans différents tissus cancéreux et normaux : valeur pronostique dans le cancer du sein
Sauter G., Schuster M., Himmler G. and Loibner H.

PEACE 2003
Expression of recombinant antibodies using a tri-cistronic expression system
M. Schuster, G. Waxenecker, G. Himmler, I. Froihofer, C. Schwager, R. Ohler and H. Loibner, igeneon

ISBT 2003
ANALYSIS OF THE SPECIFICITY OF THE HUMORAL IMMUNE RESPONSE INDUCED BY CANCER VACCINE IGN101
Manfred Schuster, Gottfried Himmler, Hans Loibner, Irmgard Froihofer, Cornelia Schwager, Helga Klug, Susanne Wiederkum, Alois Jungbauer, Astrid Duerauer and Rainer Hahn

Treatment of Breast Cancer Patients with the Cancer Vaccine IGN101 that Induces an Immune Response against the Pan-Carcinoma Glycoprotein EpCAM
Hellmut Samonigg, Hans Loibner, Marija Balic, Guenter Hofmann, Manfred Schuster and Gottfried Himmler

AACR 2004, ISBT 2004
Increased effector functions of a monoclonal antibody by glycoform engineering
M. Schuster, P. Umana1, P. Brünker1, I. Froihofer, S. Wiederkum, C. Schwager, H. Klug, G.C. Mudde, G. Himmler and H. Loibner

Annex 4 – Publications

Inhibition of Xenograft Tumor Growth and Down-Regulation of ErbB Receptors by an Antibody Directed against Lewis Y Antigen
Hesso Farhan, Christian Schuster, Markus Klinger, Eva Weisz, Günter Waxenecker, Manfred Schuster, Veronika Sexl, Geert C. Mudde, Michael Freissmuth, and Ralf Kircheis; The Journal of Pharmacology and experimental Therapeutics 2006; 319:1–8.

Compensation of endogenous IgG mediated inhibition of antibody-dependent cellular cytotoxicity by glyco-engineering of therapeutic antibodies
Andreas Nechansky, Manfred Schuster, Wolfgang Jost, Petra Siegl, Susanne Wiederkum, Gilbert Gorr, Ralf Kircheis; Molecular Immunology 2006.

Cancer Immunotherapy, review article
Manfred Schuster, Andreas Nechansky, Hans Loibner and Ralf Kirchels; Biotechnology Journal 2006; 1, 138-147.

In vivo glyco-engineered antibody with improved lytic potential produced by an innovative nonmammalian expression system
Manfred Schuster, Wolfgang Jost, Geert C. Mudde, Susanne Wiederkum, Cornelia Schwager, Evelyne Janzek, Friedrich Altmann, Johannes Stadlmann, Christian Stemmer and Gilbert Gorr, Biotechnology Journal 2007

Method for determining humoral response to active immunization with monoclonal antibody against EpCAM using peptide arrays prepared by SPOT technology
Dürauer A., Berger E.,Schuster M., Wasserbauer E., Mudde G., Himmler G., Jungbauer A., Manuscript accepted to J. Immunol. Methods

Expression of recombinant antibodies using a tri-cistronic expression system.
Schuster M, Waxenecker G, Himmler G, Loibner H; Manuscript in preparation

Increased effector functions of a therapeutic monoclonal Le-Y specific antibody by glycoform engineering.
Schuster M, Umana P, Waxenecker G, Wiederkum S, Schwager C, Himmler G, Loibner H, Mudde G.C; Cancer Research 2005; Sep 1;65(17):7934-41.65 (17).

Two novel additives for serum and serum-free media increase activity of in-vitro mammalian cells.
Karlheinz Landauer, Lucia Strommer, Manuela Kainer, Otto Doblhoff-Dier, Manfred Schuster, Gottfried Himmler, Hans Loibner, Günter Waxenecker, submitted to Biotechnology Process

Expression and purification of homogenous proteins in Saccharomyces cerevisiae based on ubiquitin-FLAG fusion.
Einhauer A, Schuster M, Wasserbauer E, Jungbauer A
Protein Expr Purif 2002 Apr 24:3 497-504

Transmembrane-sequence-dependent overexpression and secretion of glycoproteins in Saccharomyces cerevisiae.
Schuster M, Wasserbauer E, Aversa G, Jungbauer A
Protein Expr Purif 2001 Feb 21:1 1-7

High speed immuno-affinity chromatography on supports with gigapores and porous glass.
Schuster M, Wasserbauer E, Neubauer A, Jungbauer A
Bioseparation 2000 9:5 259-68

Protein expression in yeast; comparison of two expression strategies regarding protein maturation.
Schuster M, Einhauer A, Wasserbauer E, Sussenbacher F, Ortner C, Paumann M, Werner G, Jungbauer A
J Biotechnol 2000 Dec 28 84:3 237-48

Short cut of protein purification by integration of cell-disrupture and affinity extraction.
Schuster M, Wasserbauer E, Ortner C, Graumann K, Jungbauer A, Hammerschmid F, Werner G
Bioseparation 2000 9:2 59-67

Protein expression strategies for identification of novel target proteins.
Schuster M, Wasserbauer E, Einhauer A, Ortner C, Jungbauer A, Hammerschmid F, Werner G
J Biomol Screen 2000 Apr 5:2 89-97

Solvent production by Clostridium beijerinckII NRRL B592 growing on different potato media.
Nimcevic D, Schuster M, Gapes JR
Appl Microbiol Biotechnol 1998 Oct 50:4 426-8

Annex 5 – Inventions and Patent applications

1998
Integration of cell-disrupture and affinity extraction as one step procedure for purification of intracellularly expressed proteins / SW 698
Schuster M, Wasserbauer E

2000
Production and use of protein-carbohydrate conjugates / ID 2000-001
Eckert H., Loibner H., Schuster M., Himmler, G Waxenecker G.
Patent number WO 03/097663, May 15, 2002"Multiepitope Vaccine"AT, PCT

2001
EpCAM mimotope vaccine / ID 2001-001
Loibner H., Himmler G., Waxenecker G., Schuster M., Putz T.
Patent number WO 04/091655, Apr 17, 2003-05-14"Recombinant immunogenic antibody"AT, PCT

IgG2a modified immunoglobulin / ID 2001-002
Loibner H., Himmler G., Waxenecker G., Schuster M., Putz T.
Patent number WO 04/091655, Apr 17, 2003"Recombinant immunogenic antibody"AT, PCT

2002
Tricistronic expression product / ID 2002-002

Loibner H., Himmler G., Waxenecker G., Schuster M.
Patent number WO 04/091655, Apr 17, 2003"Recombinant immunogenic antibody"AT
Magnetic beads formulation / ID 2002-003
Himmler G., Loibner H., Schuster M., Wasserbauer E., Eckert H., Doblhoff-Dier O., Kircheis R., Waxenecker G.
Patent number WO 02/080966, Mar 23, 2001"Autovac II"AT, PCT
Multicompartment Electrophoresis / ID 2002-006
Himmler G., Schuster M., Waxenecker G., Wasserbauer E.
EpCAM fragment / ID 2002-011
Loibner H., Himmler G., Schuster M.
A modular ELISA for simultaneous quantitation / ID 2002-021
Nechansky A., Schuster M., Waxenecker G., Himmler G., Mudde G.

2003

Multi-epitope vaccine containing a SialylTn carbohydrate conjugate / ID 2003-003
Kircheis R. Schuster M. Himmler G. Loibner H
Patent number WO 03/097663, May 15, 2003"Multiepitope Vaccine"PCT
EpCAM epitopes / ID 2003-005
Loibner H. Himmler G. Jungbauer A. Wasserbauer E. Schuster M. Hahn R. Dürauer A.
Patent number WO 04/106917, Jun 02, 2003 "Selection of epitopes for immunotherapy" AT
CIM Discs / ID 2003-006
Loibner H. Waxenecker G. Wasserbauer E. Schuster M.
Low dose vaccine / ID 2003-015
Loibner H. Himmler G. Schuster M.
Patent number EP 04450149.2, Jul 2004, "Low dose IGN101", EP
VEGF as target for passive immuno therapy / ID 2003-016
Loibner H. Schuster M. Waxenecker G.

2004

Antibodies with specific glycosylation and antigenic surface structure / ID 2004-005
Waxenecker G Himmler G Loibner H Landauer K Schuster M Kircheis R
Combination therapy passive/active / ID 2004-007
Loibner H Schuster M
Novel Lewis y antibody / ID 2004-009
Schuster M, Himmler G, Waxenecker G, Mudde G, Loibner H, Loidl M, Redl G
Patent number PCT/EP2004/007787, Jul 14, 2004 "Modified glycosylated antibody" PCT
Increase of targeted cytotoxicity / ID 2004-010
Waxenecker G, Kircheis R, Schuster M, Himmler G
US prov.
Atomadsorptionspectrometry / ID 2004-011
Chabicowsky M Obwaller A Schuster M Szolar O

2005

Obesity treatment / ID 2005-001
Schuster M, Nechansky A, Wasserbauer E, Kircheis R

2006

DREAM inhibitors
Schuster M, Loibner H, Stranner S
Characterization of enzymatic activity in complex matrices
Schuster M, Loibner H, Janzek E
Glycoenginnered antibodies
Schuster M, Gorr G, Nechansky A., Kricheis R.
Ex vivo silencing technology
Loibner H, Schuster M

EXHIBIT 2

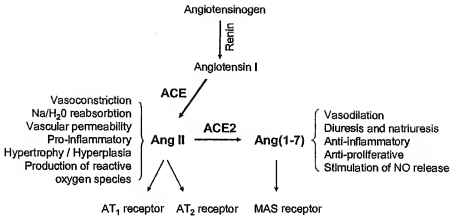


Figure 1. Simplified diagram of the Renin-Angiotensin System; roles of Ang II and Ang(1-7).

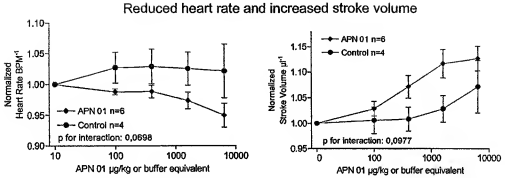


Figure 2A: Left: Following treatment with increasing doses of APN 01, the heart rate decreases; Right: Following treatment with increasing doses of APN 01, the stroke volume increases.

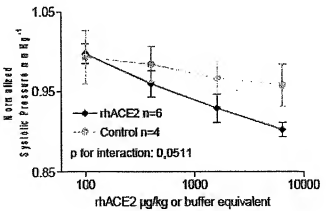


Figure 2B: Following treatment with increasing does of APN 01, the systolic pressure decreases.

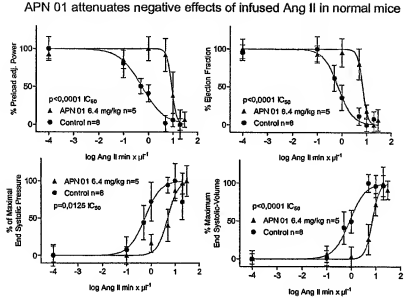


Figure 3: Pre-treatment with APN 01 significantly attenuates the negative cardiac effects of increasing doses of continuously infused Ang II.

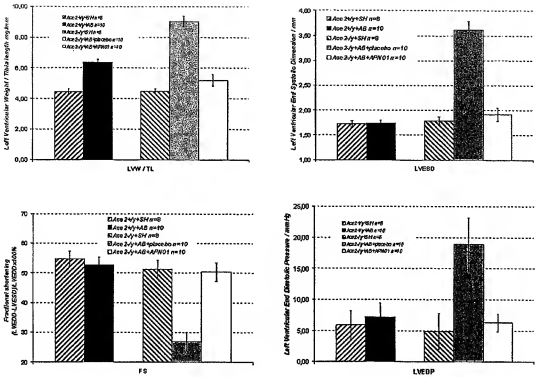


Figure 4: The upper left chart shows the effects on left ventricular weight. The upper right chart shows the effects on left ventricular end systolic dimension (LVESD). The lower left chart shows the fractional shortening. The lower right shows the left ventricular end diastolic pressure. Effects of treatment with APN 01 for all shown parameters are significant with $p < 0.01$.

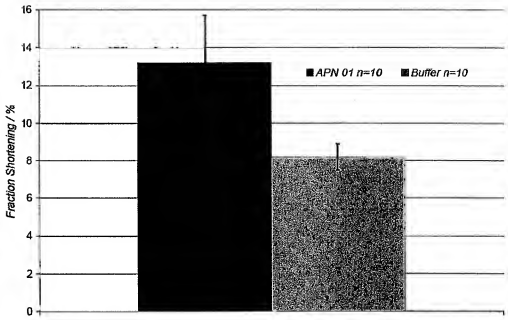


Figure 5: Fraction shortening measured by ultrasonic cardiac echography in an ischemic-reperfusion model in Balb-c mice: Investigation of therapeutic effects of APN 01 treated animals (dark-shaded bar) compared to control animals (light-shaded bar).

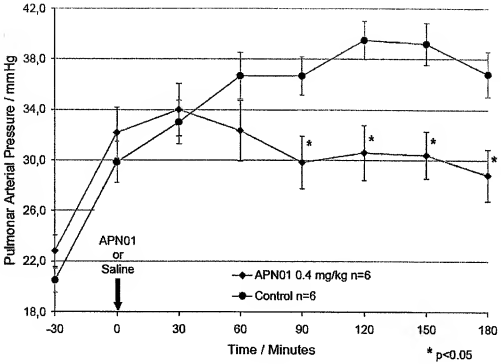


Figure 6: Pulmonary arterial pressure measured in a hypoxia induced pulmonary hypertension model in piglets. APN01 treated animals were compared to control animals.

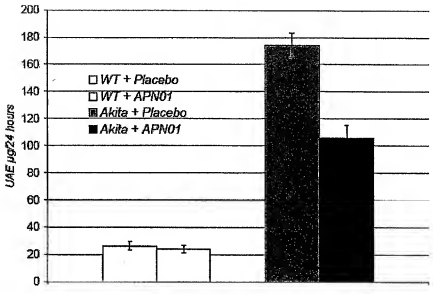


Figure 7: Reduction of albuminuria in diabetic mice by treatment with APN01.

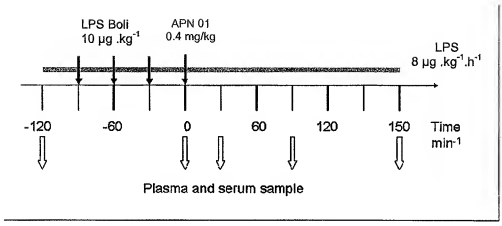


Figure 8: LPS and APN 01 dosage scheme. LPS and APN 01 administration as well as plasma and serum sampling time points are indicated.

Group	*Animal numbers*	*Average weights / kg^{-1}*	*Cumulative LPS quantity / $\mu g x kg^{-1}$*
Evaluable animals APN 01	23, 26, 28, 31, 35, 37	22.0±0.7	39.4±6.7
Evaluable animals control	6, 8, 22, 30, 33, 38	22.3±1.6	48.7±3.4
All animals APN 01	23, 26, 28, 31, 32, 34, 35, 37, 39, 41, 69	21.9±0.8	39.8±5.8
All animals Control	6, 8, 17, 22, 24, 25, 29, 30, 33, 38, 40, 42, 50, 55, 79	21.5±0.8	42.8±4.6

Figure 9: Group assignment, animal body weights and LPS administration.

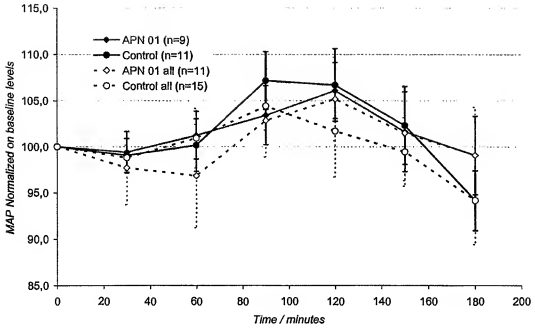


Figure 10: Graphical representation of mean arterial pressures (MAP) in an LPS induced sepsis model in piglets of animals treated with APN 01 compared to Placebo treated animals. Survivors of the whole experiment are represented in solid, the cohort of all individuals in dashed lines.

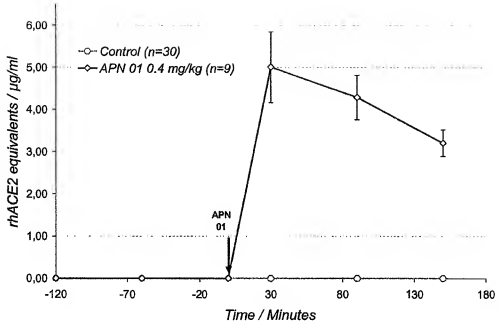


Figure 11: rhACE2 serum titers in a LPS induced ARDS model in piglets: 0.4 mg/kg APN 01 were administrated at 0 min and compared to a control group.

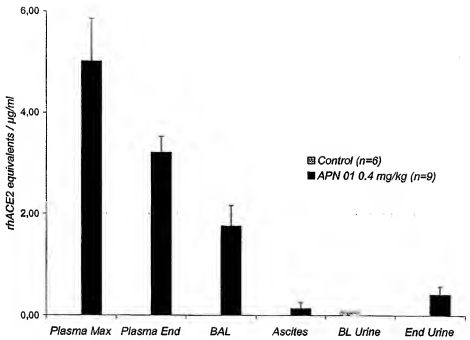


Figure 12: rhACE2 titers in body fluids in an LPS induced ARDS model in piglets: 0.4 mg/kg APN 01 were administrated at 0 min and compared to a control group.

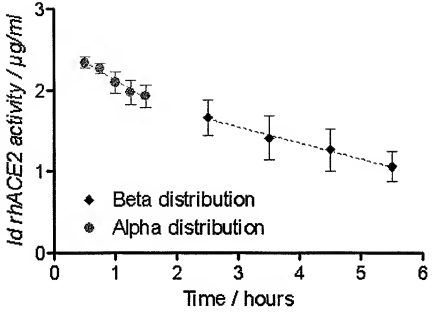


Figure 13: ld representation of rhACE2 plasma titers piglets (n=3): 0.4 mg/kg were administrated at time point 0, ACE2 activity data are displayed and separated into alpha- and beta-kinetics.

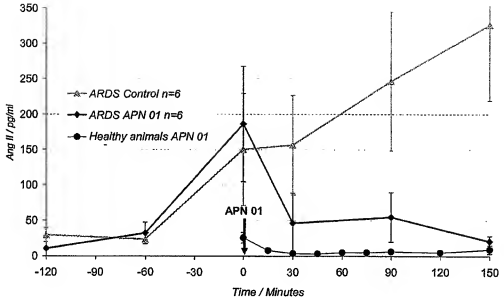


Figure 14: Graphical representation of Ang II plasma titers in piglets in an LPS induced ARDS model. Animals receiving at 120 minutes after LPS administration (time point 0 on the graph) 400 μg/kg rhACE2 were compared to a vehicle only group. Ang II titers from three healthy piglets also receiving at the 0 minutes time point 400 μg/kg ACE2 are also displayed.

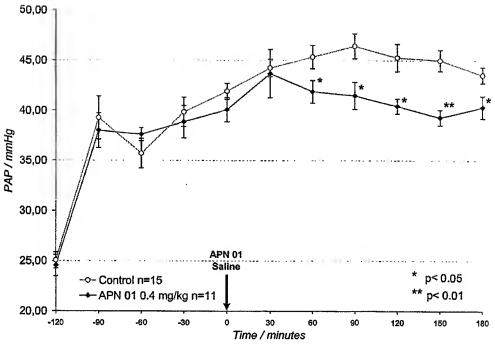
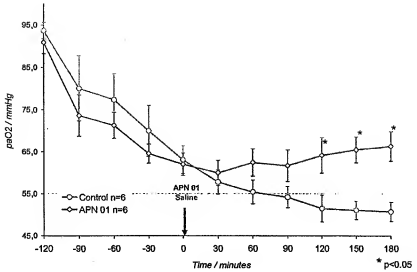
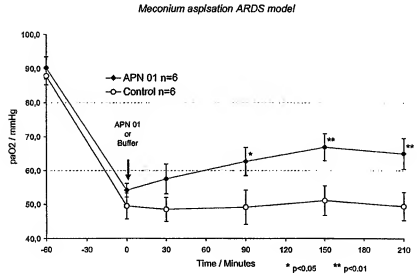


Figure 15: Pulmonary arterial pressure was monitored during the whole study. Average values of the APN 01 treated group and the control group are shown.

A



B



Figures 16A and 16B: Figure 16A shows arterial oxygen tension measured in blood samples of LPS-induced ARDS animals treated with APN 01 and control animals. Figure 16b shows arterial oxygen tension measured in blood samples of meconium apsiration-induced ARDS animals treated with APN 01 and control animals.

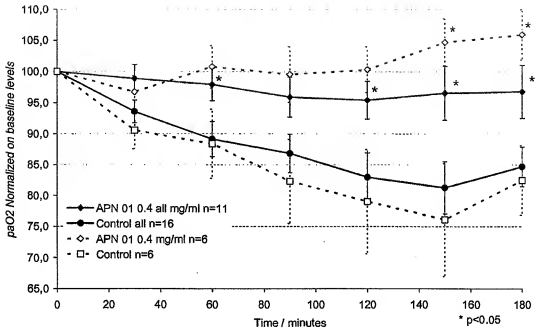


Figure 17: Arterial oxygen tension normalized on individual baseline measured in blood samples of animals treated with APN 01 and control animals. Solid curves represent data including all animals.

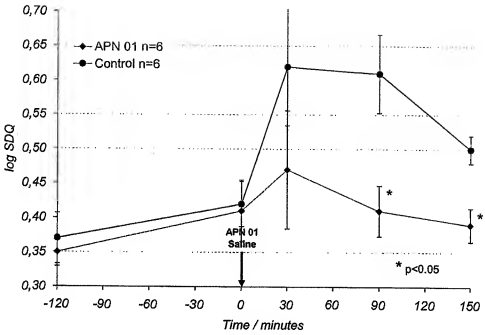


Figure 18: Graphical representation of log SDQ values calculated for APN 01 treated animals in comparison to a control group.

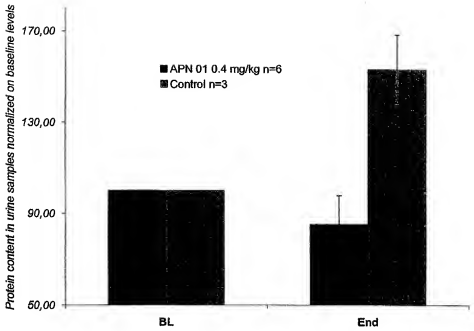


Figure 19: Comparison of protein content in urine samples taken at the end of the study in relation to baseline levels. APN 01 treated animals are displayed in dark-shaded bars, the control group animals in light-shaded bars.

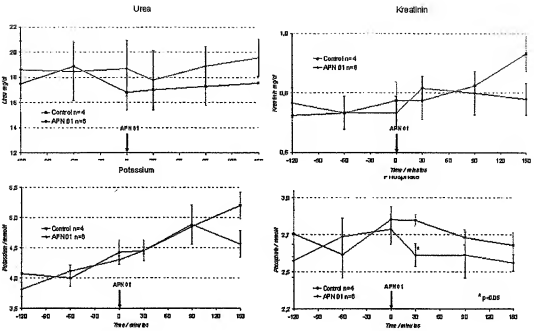


Figure 20: Comparison of urea, creatinin, potassium and phosphate content in urine samples of APN 01 treated animals and control animals.

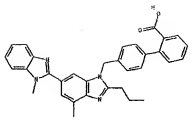


Figure 21a: Structure of Telmisartan.

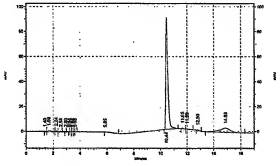


Figure 21b: RP-HPLC analysis of neutralized, aqueous Telmisartan solution stabilized with Mannitol.

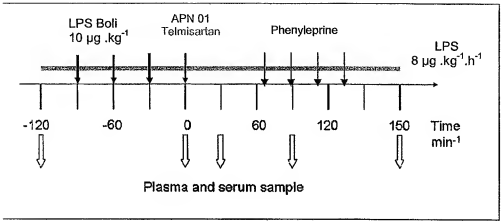


Figure 22: LPS and APN 01 dosage scheme. LPS and APN 01 administration as well as plasma and serum sampling time points are indicated.

Group	*Anima numbers*	*Average weights / kg^{-1}*	*Cumulative LPS quantity / $\mu g x kg^{-1}$*
Telmisartan	47, 49, 52, 54, 57, 59, 60, 63, 64, 73, 76, 77, 78, 82	22.0±0.7	36.2±4.7
Telmisartan + APN 01	67, 68, 70, 72, 75, 80, 81, 83	22.1±1.6	30.8±4.0
Control*	6, 8, 17, 22, 24, 25, 29, 30, 33, 38, 40, 42, 50, 55, 79	21.5±0.8	42.8±4.6
APN 01*	26, 28, 31, 32, 34, 35, 37, 39, 41, 69	21.9±0.8	39.4±6.7
Telmisartan Control	66	20.0	Ø

* From the Study Described in Paragraphs 17-37 of this Declaration

Figure 23: Animal disposition.

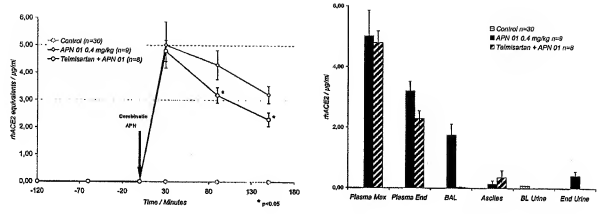


Figure 24: APN 01 PK ad PD in serum samples (left) and in several body liquids (right).

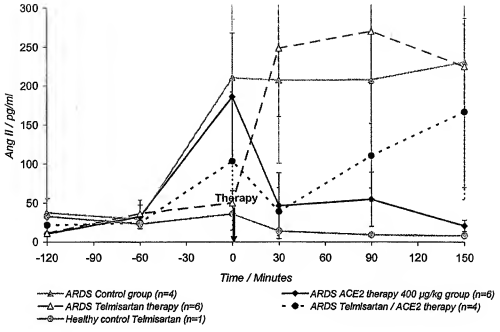


Figure 25: Ang II titers measured in plasma samples of one healthy animal treated with 1mg/kg Telmisartan in comparison to animals out of an LPS induced ARDS model receiving 400 µg/kg APN 01, 1 mg/kg Telmisartan, both Telmisartan and APN 01 or saline.

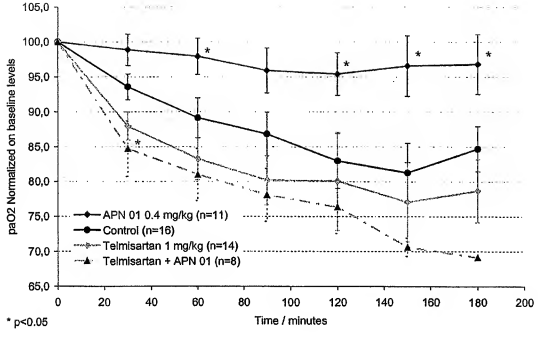


Figure 26: paO_2 measured in serum of an animal treated with 1mg/kg Telmisartan in comparison to animals receiving 0.4 mg/kg APN 01, both Telmisartan and APN 01 or saline.

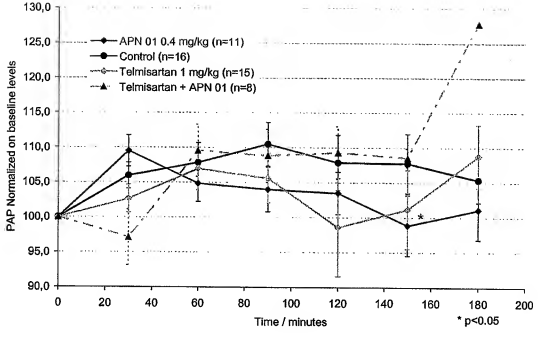


Figure 27: PAP measured for animal treated with 1mg/kg Telmisartan in comparison to animals receiving 400 µg/kg APN 01, both Telmisartan and APN 01 or saline.

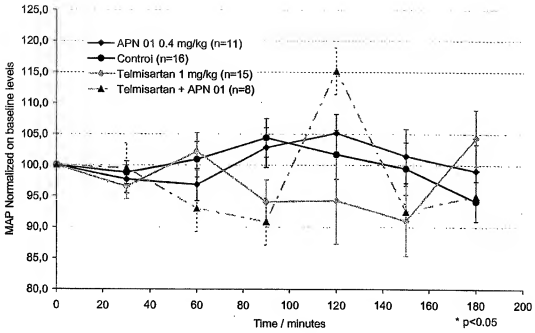


Figure 28: PAP measured for animal treated with 1mg/kg Telmisartan in comparison to animals receiving 400 µg/kg APN 01, both Telmisartan and APN 01 or saline.

Acknowledgement Receipt

The USPTO has received your submission at **15:22:41** Eastern Time on **01-NOV-2007** .

$ **1270** fee paid by e-Filer via *RAM* with Confirmation Number: 606.

eFiled Application Information

EFS ID	2413498
Application Number	10518599
Confirmation Number	8087
Title	Ace2 activation for treatment of heart, lung and kidney disease and hypertension
First Named Inventor	Joseph M. Penninger
Customer Number or Correspondence Address	32425
Filed By	Travis Wohlers/Melissa Centeno
Attorney Docket Number	SONN:064US
Filing Date	31-MAY-2005
Receipt Date	01-NOV-2007
Application Type	U.S. National Stage under 35 USC 371

Application Details

Submitted Files	Page Count	Document Description	File Size	Warnings
SONN064US_RCE.pdf	1	Request for Continued Examination (RCE)	76635 bytes	△ WARNINGS
This is not a USPTO supplied RCE SB30 form.				
SONN064US_AMENDMENTROA.pdf	24		1049511 bytes	◈ PASS

Document Description	Page Start	Page End
Amendment After Final	1	1
Claims	2	5
Applicant Arguments/Remarks Made in an Amendment	6	24

SONN064US_DECLARATION.pdf	65	Amendment Submitted/Entered with Filing of CPA/RCE	2250664 bytes	◈ PASS
SONN064US_PetitionforEOT.pdf	1	Extension of Time	61579 bytes	◈ PASS
fee-info.pdf	2	Fee Worksheet (PTO-06)	8368 bytes	◈ PASS

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date

shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

If you need help:

- *Call the Patent Electronic Business Center at (866) 217-9197 (toll free) or e-mail EBC@uspto.gov for specific questions about Patent e-Filing.*
- *Send general questions about USPTO programs to the USPTO Contact Center (UCC) .*
- *If you experience technical difficulties or problems with this application, please report them via e-mail to Electronic Business Support or call 1 800-786-9199.*